

10/701,550 filed 11/05/2003
Nikiforov et al.
O.A. Reply dated June 12, 2006

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A method of measuring the activity of a kinase enzyme, comprising:

providing a reaction mixture comprising a fluorescently labeled phosphorylatable compound, a kinase enzyme and a phosphate donor group, wherein the kinase enzyme is capable of transferring a phosphate from the phosphate donor group to the phosphorylatable compound to produce a phosphorylated product;

contacting the phosphorylated product with a ~~molecule-protein~~ having multivalent metal cations a chelating group associated therewith, and a multivalent metal ion chelated by the chelating group; and

determining a level of phosphorylated product by detecting a level of fluorescent intensity emitted from the reaction mixture.

2 (original) The method of claim 1, wherein the compound comprises a serine, tyrosine, or threonine substrate.

3. (original) The method of claim 1, wherein the multivalent metal cations bind the molecule to the phosphorylated product at least partially because of a difference in charge between the phosphorylated product and the multivalent metal cations.

4. (original) The method of claim 1, wherein the multivalent metal cations bind the molecule to the phosphorylated product at least partially because of a specific binding affinity between the metal cations and a phosphate group associated with the phosphorylated product

5. (original) The method of claim 1, wherein the multivalent metal cations comprise trivalent metal cations.

10/701,550 filed 11/05/2003
Nikiforov et al.
O.A. Reply dated June 12, 2006

6. (original) The method of claim 5 wherein the trivalent metal cations comprise Fe^{3+} .

7. (original) The method of claim 1, further comprising introducing at least a first test compound into the reaction mixture and comparing the level of fluorescent intensity emitted from the reaction mixture in the presence of the test compound to the level of fluorescent intensity emitted from the reaction mixture in the absence of the test compound.

8. (original) The method of claim 7, further comprising repeating the providing, introducing and comparing steps with a plurality of different test compounds.

9. (original) The method of claim 1, wherein the molecule comprises a polymer.

10. (withdrawn) A method of measuring the activity of a phosphatase enzyme, comprising:

providing a reaction mixture comprising a fluorescently labeled phosphorylated compound, a phosphatase enzyme, and a molecule having multivalent metal cations associated therewith; and

determining a level of dephosphorylated product produced by the activity of the phosphatase enzyme by detecting a level of fluorescent intensity emitted from the reaction mixture.

11 (withdrawn) The method of claim 10, wherein the fluorescent intensity increases in proportion to the amount of dephosphorylated product in the reaction mixture.

12. (withdrawn) The method of claim 10, wherein the multivalent metal cations comprise trivalent metal cations.

13. (withdrawn) The method of claim 12, wherein the trivalent metal cations comprise Fe^{3+} .

10/701,550 filed 11/05/2003
Nikiforov et al.
O.A. Reply dated June 12, 2006

14. (withdrawn) The method of claim 10, further comprising introducing at least a first test compound into the reaction mixture and comparing the level of fluorescent intensity emitted from the reaction mixture in the presence of the test compound to the level of fluorescent intensity emitted from the reaction mixture in the absence of the test compound.

15. (withdrawn) The method of claim 14, further comprising repeating the providing and comparing steps with a plurality of different test compounds.

16. (withdrawn) The method of claim 10, wherein the molecule comprises a polymer.

17. (Currently Amended) A method of monitoring the activity of an enzyme, comprising:

providing a first mixture comprising a fluorescently labeled substrate and an enzyme, wherein the enzyme is capable of modifying the chemical structure of the substrate to produce a fluorescently labeled product;

contacting the product with a molecule-protein having multivalent metal cations a chelating group associated therewith, and a multivalent metal ion chelated by the chelating group; and

determining a level of product produced by the activity of the enzyme by measuring binding of the molecule to the product.

18. (previously presented) The method of claim 17, wherein the enzyme is capable of modifying the chemical structure of the substrate by addition to, subtraction from, or alteration of its chemical structure.

19. (previously presented) The method of claim 17, wherein the substrate comprises a serine, tyrosine, or threonine substrate.

10/701,550 filed 11/05/2003
Nikiforov et al.
O.A. Reply dated June 12, 2006

20. (previously presented) The method of claim 17, wherein the multivalent metal cations bind the molecule to the product based at least partially on a difference in charge between the product and the multivalent metal cations.

21. (previously presented) The method of claim 17, wherein the multivalent metal cations bind the molecule to the product based at least partially on an affinity between the metal cations and a phosphate group associated with the product

22. (previously presented) The method of claim 17, wherein the multivalent metal cations comprise trivalent metal cations.

23. (previously presented) The method of claim 22, wherein the trivalent metal cations comprise Fe^{3+} .

24. (previously presented) The method of claim 17, wherein the substrate comprises a phosphorylated substrate and the enzyme comprises a phosphatase enzyme.

25. (previously presented) The method of claim 17, wherein the substrate comprises amino or keto containing substrate and the enzyme comprises an amino transferase.

26. (previously presented) The method of claim 17, wherein the substrate includes a substrate for one of the following: a sulfatase, a phosphorylase, an esterase, a hydrolase, an oxidase, or an analog thereof.

27. (previously presented) The method of claim 17, wherein the determining step is performed using fluorescence polarization detection.

28. (previously presented) The method of claim 17, wherein the determining step is performed using fluorescence intensity detection.